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A NEW METHOD OF ADDING CRESOL TO ANTITOXINS AND ANTISERUMS

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As is well known, the addition of cresol to antitoxics and other antiserums results in the formation of a precipitate due to the combination of the cresol with the proteins. This precipitate is greatest when all the cresol needed to give the desired concentration is added rapidly. To avoid this more or less voluminous precipitate, various methods have been resorted to. The cresol may be added in a dilute form. This method has the disadvantage of diluting the product to which it is added. Another method is to add the undiluted cresol drop by drop to the product. In this method the serum is commonly placed in an open vessel and beaten with a rod or paddle as the cresol is added to obtain thorough mixing. Similarly, admixture is often accomplished by adding the cresol drop by drop to the large stock bottles, replacing the cork and shaking the container thoroughly after each addition. These methods are not above reproach, as they open the way to contamination. Different producers have devised various methods to limit the exposure to contamination during the addition of the preservative. It is evidently essential that the amount of precipitate formed should be limited as much as possible. In the first place, the precipitate binds up the preservative and thus lessens the amount available throughout the product. How much remains in the serum is not known. Furthermore, the precipitate increase the loss of the product in the process of filtration and add as well to the difficulty of this process.

These considerations raised the question whether we could not find a better method for the addition of cresols to antitoxins and serums. We considered the possibility of utilizing stable emulsions using menstruums other than water, in which emulsions separate quickly. Solvents other than water in which the solubility of cresol is low, were also considered. After several preliminary experiments, ether was tried as a solvent. It was found that equal parts of cresol and ether are miscible and make a permanently clear solution. On the addition of such a mixture to antitoxin or serum, the solution floats on the

Received for publication, Feb. 7, 1921.

surface causing a slight haze at the point of contact. If shaken immediately after the addition either no precipitate or at most very little is formed. Even the rapid addition of all the solution needed to give a concentration of 0.4% to 0.5% of cresol, causes at most only a slight precipitation; the amount of which is in no way comparable to that resulting from the rapid or even drop by drop addition of the same amount of cresol alone.

With this success in eliminating precipitation when the whole volume of preservative was quickly added, several questions immediately arose: First, what would be the effect of the additional ether when the product is administered? Second, would the product, on aging, precipitate, or in other words would the cresol slowly but eventually cause as much precipitate as if it alone had been added at first? Third, would the loss of ether during filtration and other processes result in objectionable precipitate? Fourth, would the bactericidal action of cresol be decreased by the ether or not? If not, would the additional bactericidal action of the ether be of value? Fifth, would the ether affect the potency of the product?

We are indebted to Dr. Gwathmy for an opinion as to the probable harmlessness of the ether, in the amounts under consideration, when given intraspinously or intravenously. The intraspinous injection of antimeningococcus serum with the ether-cresol preservative was first tried with a monkey. As there was no evidence of pain or untoward after effects, the product was then tried in cases of cerebrospinal meningitis by Dr. Josephine Neal and her associates. They could see no difference between the serum thus preserved and that containing cresol only.

We have been unable thus far to obtain similar trials of the intravenous injection of antipneumococcus serum with the combined preservative. However, Dr. Russell Cecil has added 0.4 c c of ether per dose of antibody solution of Huntoon and finds no evident ether effect, when this is given intravenously. We see no reason, therefore, for hesitancy in using serum containing the combined preservative. At the Willard Parker Hospital, diphtheria antitoxin containing this preservative has been utilized for intravenous injections. With the doses employed, the amount of ether is small and no ill effect is noted. One of us (E. J. B.) had observed, in conjunction with Dr. C. A. Thompson of the hospital staff, that chilliness or actual chills were more common when the intravenously administered antitoxin showed cloudiness or a slight precipitate. For the last few months a preparation to which ether-cresol had been added has been in use and although this preparation is now cloudy chills are not induced. Another preparation, however, containing cresol only, but which is very clear, has just been tried and causes chills. The addition of 0.4% of ether to this product does not lessen these reactions. The conditions in these two preparations were not quite the same, as in the second preparation the ether was added separately. These experiences would seem to suggest that ether, under certain conditions may influence such reactions. A further study of this subject under comparable conditions seems indicated.

Experience shows that the subcutaneous injection of antitoxins containing ether-cresol are not rendered more painful by the presence of the ether. The use of ether in hypodermic therapy would lead one to anticipate this.

As to the subsequent development of precipitation in antitoxins and serums to which ether-cresol has been added, we can at least say that it is no greater than had cresol been added alone. In the case of antitoxic globulins, an example may be cited of a lot taken from the dialyzing bags and filtered through paper pulp to clarify it. Ether-cresol was then added. At most, only a slight opalescence developed and this did not increase nor did an appreciable precipitate form even after several months in the ice chest.

To test the effect on serums, a bleeding was taken and split into three parts. To one part was added cresol, to another, cresol-ether, and no preservative to the third. The following tabular summary gives the degrees of precipitation observed in samples filtered at increasing intervals after the addition of the preservative.

Serum. Preservative added 3/13/20.

Successive Samples, Filtered	Relative Amount of Precipitate 11/5/20		
	Cresol	Cresol-Ether	No Preservative
4/ 9/20	+	+	++
4/23/20	+	+	(not done)
6/ 3/20	+	±	++
10/30/20	—	—	Trace

Symbols ± to ++, relative amounts of precipitate; —, none.

In these filtered samples, the precipitation progressed about equally in the cresol and ether-cresol samples but more rapidly in the sample without preservative. The last was not due to bacterial contamination as was shown by cultural tests.

In the original unfiltered samples there was a copious precipitate in the sample without preservative, about half this amount of precipitate in the sample containing cresol alone. The sample containing ether-cresol had less than one-tenth the precipitate present in the cresol sample. This would be expected, as the precipitate caused by the addition of the cresol would be present in the cresol sample, as well as the precipitate which subsequently formed. It is of interest to note that the greatest precipitation on aging was encountered with the serum containing no preservative.

With two other samples of serum to which the preservatives were added on March 17, 1920, and which were filtered April 15, 1920, the precipitation observed Nov. 5, 1920, was more marked in the case of the sample to which the cresol alone was added.

The amount of ether lost during the processes of filtration, that is, filtration through paper pulp and through the Berkefeld filter, has not resulted in the formation of a precipitate during the stages of the process or as it finally comes from the Berkefeld filter. The filtered product is crystal clear. The amount of ether is small and is most probably in true solution. There can be little doubt of this as ether is soluble in water up to practically 10%. We have gained the impression that when serum is saturated with ether evaporation of the ether becomes progressively more difficult as the percentage left decreases. Even prolonged heating will not rid the serum of the ether odor. If this impression is correct, it would indicate that of the slight amount (0.4%) added to the serum, little is lost during the process of filtration.

In regard to the fourth question, namely, would the ether lessen the bactericidal action of the cresol or would it leave it unchanged, and if the action of the cresol were unchanged, would the bactericidal action of the ether make the mixture appreciably more active, the appended tables of the disinfectant tests show that the mixture is more efficacious than cresol alone.

We expected this because of some previous experiences with tetanus antitoxin. We have from time to time, as have others, encountered antitoxins and other products which are contaminated with a cresol resistant bacterium (that is, one resistant to the amount of cresol used). This bacterium passed through the filter in small numbers so that the sterility tests immediately after filtration often failed to reveal their presence. After several weeks' storage, however, the product would again reveal this contaminant due apparently to some multiplication of the bacterium in spite of the cresol.

TABLE 1
COMPARATIVE BACTERICIDAL ACTION OF CRESOL AND OF CRESOL DISSOLVED IN ETHER
Tested 2/26/20

Sample	Dilution*	Time of Exposure		
		2½ Minutes	5 Minutes	7½ Minutes
Cresol	1:200	—	—	—
	1:250	—	—	—
	1:300	+	+	—
Cresol dissolved in ether	1:200	—	—	—
	1:250	—	—	—
	1:300	+	—	—

Hygienic Laboratory Standard Method.
— = no growth, + = growth, 48 hrs. 37 C. readings.
* The dilutions refer to the dilution of the cresol. As the cresol was dissolved in an equal quantity of ether, the dilutions under "cresol dissolved in ether" indicate also the dilution of the ether.

TABLE 2
COMPARATIVE BACTERICIDAL ACTION OF CRESOL AND OF CRESOL DISSOLVED IN ETHER
Tested 2/28/20

Sample	Dilution	Time of Exposure		
		2½ Minutes	5 Minutes	7½ Minutes
Cresol	1:200	—	—	—
	1:250	+	—	—
	1:300	+	—	—
Cresol dissolved in ether	1:200	—	—	—
	1:250	—	—	—
	1:300	—	—	—

Hygienic Laboratory Standard Method.
— = no growth, + = growth, 48 hrs. readings.

In several instances during the war, the shortage due to this type of contamination became so acute due to the unprecedented demands that we resorted to the addition of ether. We did this in the light of the more or less general use of this method to sterilize albuminous fluids for bacteriological use. The addition of the ether quickly sterilized the antitoxin. In these instances, a proportionately large amount of ether was employed and the excess was subsequently driven off by heat.

More recently we encountered a similar contamination in a lot of toxin-antitoxin mixture. The addition of only 0.4% of ether resulted in apparent sterilization. The tests of sterility were not made until seven and eight days after the addition of the ether so that we do not know how soon sterilization was effected. This amount of ether (0.4%) has, however, failed to sterilize one lot of contaminated tetanus antitoxin.

On the whole, our experience and that of others who have tried the combined ether-cresol preservative, indicates that the increased bactericidal activity is of decided practical importance, especially in relation to those products which, because of their mode of preparation, cannot be kept sterile.

Experience has shown that the potency of serums and antitoxins is uninfluenced by the ether. Preparations of diphtheria antitoxin, antimeningococcus serum and of antipneumococcus serum have been retested as long as 7 months after addition of the ether cresol. The ether may be added to a toxin-antitoxin mixture without interfering with the balance as the ether is without effect on the more labile toxin element. Thus on Aug. 27, 0.4% of ether was added to a toxin-antitoxin mixture. This mixture was toxic, killing guinea-pigs on the fifth or sixth day. Tested 53 days later, the pigs died on the sixth day.

Certain theoretical considerations have arisen while collecting the purely practical facts outlined in the foregoing. These points may be mentioned. On mixing the ether and cresol, considerable heat is generated. We have not attempted to determine whether any new compound is formed. If the mixture consists of 25% of ether and 75% of cresol, instead of half and half, the addition to serum results in a marked opalescence of the serum although marked precipitation may not occur. Ether tends to reduce the surface tension of the serum. This may possibly be a factor not only in inhibiting precipitation but also a factor in the apparent inhibition of reaction after the intravenous administration of antitoxin. We have not attempted to give a summary of the previous use of ether as a preservative nor of the use of ether in the prevention of anaphylactic shock in experimental animals. The former is generally well known. The latter would involve a discussion which we wish to avoid by simply presenting the practical results obtained without any attempt at explanation.

SUMMARY

A mixture of equal parts of ether and cresol is presented as a new preservative for antitoxins and serums. This mixture is added in amounts necessary to give the required concentration of cresol. The addition of this mixture causes much less precipitate than does cresol

alone. Subsequent precipitation is not necessarily limited by the ether, it is never greater than that in products containing cresol alone. The mixture of ether and cresol is more strongly antiseptic than cresol alone. In therapeutic application, the ether is not a disadvantage. In the case of intravenously injected antitoxin, the indication that the ether may under certain circumstances reduce the incidence of adverse reactions warrants further comparative work. Ether may be added to the toxin-antitoxin mixture without disturbing the balance of the mixture.